Asymmetric quantum dots (QD) provide non-bleaching imaging probes yielding orientation-dependent optical signals from individual cell surface proteins. The Invitrogen QD655 measures 12.8 x 5.8 nm and exhibits an initial fluorescence anisotropy of about 0.1. Calculated rotational correlation times for rotation in water about the particle short and long axes, 0.27 μs and 0.18 μs respectively, suggest that the nanoparticle can probe molecular rotation down to the μs timescale. We have used QD655 conjugated to A2 DNP-specific IgE to explore slow rotation of the Type I Fcε receptor on variously-treated RBL-2H3 cells. We excite fluorescence from cell-bound QD with illumination polarized at 45 deg and use an image splitter and an EMCCD camera to record 100 fps image sequences containing simultaneous x- and y-polarized sub-images in each frame. Blinking of spots verifies imaging of individual QDs. Time-dependent fluorescence from regions containing individual QDs in image pairs is extracted and the time-autocorrelation function for polarization fluctuations calculated with correction for blinking-induced intensity fluctuations. Individual QDs exhibit peak polarization fluctuations with an RMS amplitude of ~0.04 which decay slowly over 100-300 ms. This behavior is exhibited on untreated cells and on cells treated with polyvalent DNP-BSA, methyl-β-cyclodextrin or cytochalasin D or fixed with paraformaldehyde. Previous time-resolved phosphorescence anisotropy (TPA) measurements showed substantial limiting anisotropies for these receptors, indicating that complete receptor orientational randomization required times beyond the 1 ms timescale of TPA experiments. Whether the slow decay observed in the present experiments represents the hindered receptor rotation implied by TPA results remains to be determined. Work on faster techniques aimed at single molecule rotation measurements in the microsecond timescale is underway. Supported by NSF grants MCB-1024668 and CHE-0628260.

3319-Pos Board B180
Optimal Diffusion Coefficient Estimation in Single-Particle Tracking
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Despite being widely and successfully applied to study transport in biophysical systems, the statistics of single-particle tracking data are only partially understood even in the simplest case of free diffusion. Here, we present the correct distribution of measurement results for a freely diffusing particle observed with localization error sigma and a finite camera integration time. We derive the fundamental limit (Cramer-Rao bound) on the error in estimating the diffusion coefficient D from such data, represented by a simple formula that can be applied to judge whether experimental data contains enough information to determine D. Two recently developed estimation procedures, a maximum-likelihood estimator [A. J. Berglund, Phys. Rev. E 82, 011917 (2010)] and an optimized least-squares fit to the mean-square displacement [X. Michalet, Phys. Rev. E 82, 041914 (2010)], are shown through numerical simulations to be nearly optimal in extracting D and sigma. These results can be applied to understand when D can be determined with reasonable confidence from short trajectories or in high-noise scenarios.

3320-Pos Board B181
The Nature of Constitutive Activation of HER2 at the Single Molecule Level
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HER2 is a highly active kinase that is distinguished from the other HER family members in that no ligand has been identified to directly bind the receptor. HER2 can be trans-activated by forming complexes with other receptors, but it also exhibits constitutive activation when it is over-expressed. In fact, this ligand-independent activation plays an important role in driving the growth of HER2 amplified tumors. To gain further mechanistic understanding of the constitutive HER2 activation, we performed single molecule tracking studies of HER2 and its mutants on the living cell membrane and developed new analysis tools. From these studies, we found that activation of HER2 is less regulated by the structural features of its ectodomain than that of EGFR as we previously demonstrated. Rather, HER2 activation may be largely related to its interaction with membrane subdomains, which in turn modulates its local density. Indeed, we found that cholesterol content and distribution pattern on the membrane altered the diffusion dynamics of HER2 and its phosphorylation status. The modulation of HER2 activation by cholesterol may have relation to tumor cell response to trastuzumab.

Auditory Systems

3321-Pos Board B182
An Active Mechanism for Signal Detection in the Mammalian Ear
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The ear's exquisite sensitivity, sharp frequency tuning, and broad dynamical range result from an active process known as the cochlear amplifier. Although outer hair cells play a central role in cochlear amplification, their mechanism of action remains uncertain. In non-mammalian ears hair bundles, the sensory organelles of hair cells, can perform mechanical work and account for the active process in vitro. Although active hair-bundle motility also occurs in the mammalian cochlea, a membrane-based piezoelectric effect known as electromotility is required for amplification in vivo.
We present a physical description of a segment of the mammalian cochlea that, by amplifying the power of an external input, acts as a sensitive, sharply tuned, and nonlinear signal detector. This model, which is based on the known physiology and morphology of the inner ear, couples active hair-bundle motility and electromotility through the geometric arrangement of hair cells in the organ of Corti. The model displays quantitative agreement with in vivo measurements of basilar-membrane movements. The model's predictions of the vibration pattern at different input levels and of the differences between wild-type and electromotility-deficient mice accord qualitatively with experimental observations.
We demonstrate how internal sources of energy in this system enhance the signal-detection properties of the mammalian ear in comparison with those of a passive cochlea. Although the hair bundle provides nonlinearity and feedback sufficient for detecting low-frequency signals in non-mammals, electromotility produces additional feedback that allows the mammalian cochlea to detect high-frequency inputs. This work supports an evolutionary scenario in which high-frequency hearing in mammals arose by supplementing a preexisting auditory amplifier with an additional source of mechanical energy.

3322-Pos Board B183
Otoacoustic Emission through Waves on Reissner's Membrane
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The cochlea not only acts as a detector of sound but can also produce tones itself. These otoacoustic emissions are a striking manifestation of the mechanical active process that sensitizes the cochlea and sharpens its frequency discrimination. It remains uncertain how these mechanical signals propagate back to the middle ear, from which they are emitted as sound. Although reverse propagation might occur through waves on the basilar membrane, experiments suggest the existence of a second component in otoacoustic emissions. We have combined theoretical and experimental studies to show that mechanical signals can also be transmitted by waves on Reissner's membrane, a second elastic structure within the cochea. We have developed a theoretical description of wave propagation on the parallel Reissner's and basilar membranes and its role in the emission of distortion products. By scanning laser interferometry we have measured traveling waves on Reissner's membrane in the gerbil, guinea pig, and chinchilla. The results accord with the theory and thus support a role for Reissner's membrane in otoacoustic emission.

3323-Pos Board B184
Magnetic Nanoparticles as Mechanical Actuators of Inner Ear Hair Cells
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Mechanical sensation by the auditory system is performed by hair cells, named in reference to the stereociliary bundles that protrude from their apical surface. They have been shown to exhibit active motility under in vitro conditions and a highly nonlinear dynamic response. To explore their mechanical properties, we developed a technique that utilizes paramagnetic beads to actuate the stereocilia. Steady-state deflections were imposed and seen to strongly affect the dynamic state of the bundle, inducing a transition from multi-mode to single-mode state, as well as the crossover from the oscillatory to the quiescent state. Numerical simulations capturing the behavior near the critical points will be presented.

3324-Pos Board B185
Effect of Non-Steroidal Anti-Inflammatory Drugs on the Outer Hair Cell Protein Prestin
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Prestin belongs to the SLC26 protein family of anion transporters. Prestin (SLC26A5) is a motor protein essential for the electromotility of the outer hair- cells (OHC) and therefore the amplification of sound in the cochlea. This protein is able to convert changes in membrane potential to mechanical force and to modify the length of the OHC. The electromotility of prestin-expressing cells is associated with a nonlinear capacitance (NLC) that can be measured electrophysiologically. Prestin is a mechanosensitive protein and its function is altered by reagents known to change membrane mechanical properties.

The non-steroidal anti-inflammatory drug (NSAID) salicylate has been showed to inhibit the NLC and the electromotility of OHC and prestin-expressing HEK. It is thought to compete with anions such as chloride for the anion-binding site on prestin. Other NSAIDs such as ibuprofen, naproxen, piroxicam or diflusinal can trigger side effects related with hearing and sometime cause tinnitus by an unknown mechanism. Here, we investigate a possible mechanism for these adverse reactions by examining the effects of these drugs on the function of prestin. The prestin-associated NLC is monitored by whole-cell as well as inside-out patches from HEK cells expressing prestin, and recorded before and after the perfusion of NSAIDs. This allows a cell-by-cell comparison of the NLC parameters in the presence and absence of the tested molecule. Ibuprofen and naproxen both showed an effect on the half-maximum voltage (V1/2) and the charge density parameters of the NLC. The effect of ibuprofen was particularly pronounced, shifting V1/2 from −70 mV to −52 mV and increasing the maximal charge movement by 30%. Further studies will contribute to our understanding of whether NSAIDs act through alteration of the mechanical properties of the membrane or specifically interfere with prestin function.

3325-Pos Board B186

Thermal Sensitivity of Vestibular Neuroepithelium in the Toadfish

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Core body temperature is known affect the static and dynamic properties of vestibular nerve afferents, although the underlying mechanisms for the differential sensitivity of the two afferents types to temperature changes are unknown. We recorded the changes in spontaneous discharge rate, regularity, adaptation to step hair bundle displacements and responses to sinusoidal head rotations of single-units to changes in temperature of the crista ampullaris in the oyster toadfish. Similar temperature gradients were introduced with perfusion of temperature-controlled artificial perilymph and with irradiation of the crista with 980 and 1860 nm light. A broad spectrum of afferent responses to thermal perfusion was observed: some neurons were insensitive to temperature, some increased their spontaneous discharge rate, and others showed a decrease in their discharge rate with temperature. The very short focused pulses of light at the two wavelengths deliver transient pulses of thermal energy (dT/dt) without large increases in accumulated temperature of cells or tissue. The irradiation of the crista produced different responses from the same afferents for a similar change in temperature. These results suggest potential wavelength specific absorption within the vestibular neuroepithelium underlies the differences between the three forms of heat delivery. Depending upon the hair cell type irradiated, the fast temperature changes induced may modulate the intracellular Ca2+ levels differently affecting the neurotransmitter release. Regardless of the mechanisms involved, the presence of temperature compensating mechanisms in the hair cell and afferent complex may allow sensitive function over a wide range of temperatures.

3326-Pos Board B187

Imaging Stereocilia Links in Live Auditory Hair Cells

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We previously imaged stereocilia links in glutaraldehyde-fixed mammalian auditory hair cells using hopping probe scanning ion conductance microcopy (HPSICM, Novak *et al*. Nat Methods, 2009). Due to the complex three-dimensional structure of the stereocilia bundle, achieving high-resolution images required a considerable amount of time (~44 min/bundle). To study the dynamics of stereocilia link regeneration in real time, we needed a significantly faster yet reliable way to continuously scan the stereocilia bundles in live hair cells.

To improve the HPSICM imaging speed, we re-designed the scan head by incorporating a faster piezo assembly with a resonant frequency of ~18 kHz for Z-movement. Despite having a less sensitive strain gauge sensor (compared to the previously used capacitive sensor), the vertical resolution of the system remained the same (~5nm). Significantly smaller inertia allowed mounting the scan head on a rotational platform and scanning the sample at any angle, a pre-requisite for the successful imaging of tip links. Moreover, the overall image resolution was slightly decreased and we can now image hair cell bundles significantly faster (~11 min/bundle).

The performance of the improved system was tested using cultured organ of Corti explants from the *Shaker 2* and *Whirler* mice due to their short stereocilia with abundant stereocilia links (typically ~5nm in diameter and ~100-300nm in length). To test the ability of HPSICM to detect these miniature structures at high imaging speed we performed continuous time-lapse scanning and looked for reproducibility of the links in consecutive images. Next, to rule out the possibility that the observed links were simply scanning artifacts, we disrupted the links by treating the explants with BAPTA-buffered Ca^{2+}-free medium. Our results demonstrate that the improved HPSICM technique successfully visualizes stereocilia links in live auditory hair cells.

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3327-Pos Board B188

Nonlinearities in Threshold-Level Detection by Inner Ear Hair Cells

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Auditory system is known for its exquisite sensitivity with sub-nanometer detection thresholds. Mechanical deflections due to external sound and ground vibrations are converted by inner ear hair cells into electrical signals. In some species, hair cell's stereociliary bundles exhibit spontaneous oscillations under *in vitro* conditions, a behavior that has been successfully reproduced by numerical models based on nonlinear dynamics. In this work, we study both numerically and experimentally the dynamics of individual hair bundles from the Bullfrog sacculus, at threshold levels of stimulation to elucidate the mechanisms underlying the sensitivity of detection by the sacculus. We measure the steady state response to small sinusoidal stimuli, as well as the time course of the phase locking transition in spontaneously oscillating bundles.

3328-Pos Board B189

Self-Tuning of Hair Cells in the Bullfrog Sacculus

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Spontaneous oscillations of the stereociliary bundle of a hair cell - the mechanosensory cell in auditory and vestibular systems - is considered to be a signature of an active amplification mechanism. We study whether an internal self-tuning process governs the active motility, by mimicking the effects of loud sound on the spontaneous oscillation. After the application of high-amplitude stimuli, with deflections on the order of micrometers applied to the hair bundle, the active oscillatory motion of the hair bundle was suppressed for hundreds of milliseconds, indicating a change in the dynamic state of the hair cell. Here we observe the recovery profile of an oscillating hair bundle after cessation of deflection. Data is compared to mathematical models which include a feedback equation to capture the temporal changes in the profile of the limit cycle oscillations.

Peptide & Toxin Ion Channels

3329-Pos Board B190

Increase in Cytotoxic Effect of Tolaasin by Phospholipids Composed of Medium-Chain Fatty Acids

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Tolaasin produced by *Pseudomonas tolaasii* disrupts membrane structures of cultivated mushrooms, such as *Agaricus bisporus* and *Pleurotus ostreatus* (oyster mushroom), and causes brown blotch disease. It consists of 18 amino acids, its molecular mass is 1,985 Da, and it forms a left-handed α-helix. The mechanism of membrane-pore formation of tolaasin molecule has not known in detail. When tolaasin molecule is inserted into the membrane, N-terminus of tolaasin forms 4 turns of α-helix and the length of tolaasin channel corresponds to near 20 Å, a little shorter than the thickness of membrane. Tolaasin channels are unstable in the artificial lipid bilayer and this may be explained by the comparison between the length of tolaasin channel and the thickness of lipid bilayer membrane. In control condition, bilayer was made with phosphatidyl